

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

**Amendments to the Claims:**

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

- 1-3. (Canceled)
4. (Withdrawn) The composition of claim 3 wherein said carrier comprises an inhibitor stabilizing medium.
5. (Withdrawn) The composition of claim 4 comprising calcium ion.
6. (Withdrawn) The composition of claim 4 comprising a steroid hormone.
7. (Withdrawn) The composition of claim 6 wherein said steroid hormone is DHT.
8. (Withdrawn) The composition of claim 4 comprising a substance that depresses the freezing point of said composition below about -20°C.
9. (Withdrawn) The composition of claim 3 comprising a steroid-hormone depleted body fluid or secretion.
10. (Withdrawn) The composition of claim 9 wherein said body fluid comprises blood plasma or serum.
11. (Canceled)
12. (Withdrawn) The inhibitor of claim 11 wherein said at least one said immunoglobulin is a secretory immunoglobulin.
13. (Withdrawn) The inhibitor of claim 11 wherein said at least one immunoglobulin is chosen from the group consisting of IgA, IgM and IgG, and combinations thereof.

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

14. (Withdrawn) The inhibitor of claim 13 wherein said IgA is dimeric or polymeric.
15. (Withdrawn) The inhibitor of claim 13 wherein said IgM is polymeric.
16. (Withdrawn) The inhibitor of claim 13 wherein at least one immunoglobulin is chosen from the group consisting of IgG1 $\kappa$  and IgG2.
17. (Withdrawn) A steroid hormone irreversible cell growth inhibitor composition comprising at least one immunoglobulin inhibitor that is active with respect to the ability to inhibit steroid hormone-responsive cancer cell proliferation and inactive with respect to steroid hormone reversibility of said inhibition, and a carrier.
18. (Withdrawn) A method of making a steroid hormone irreversible cancer cell growth inhibitor composition comprising exposing the inhibitor composition of claim 3 to calcium depleted conditions for a defined period of time sufficient to render said at least one immunoglobulin inhibitor irreversibly inhibitory of steroid hormone responsive cancer cell growth *in vitro*.
19. (Withdrawn) An immunoglobulin inhibitor mimicking substance, said immunoglobulin inhibitor comprising at least one secretory immunoglobulin chosen from the group consisting of IgA, IgM and IgG having activity for steroid hormone reversably inhibiting steroid hormone responsive cancer cell growth *in vitro*.
20. (Withdrawn) The mimicking substance of claim 19 comprising tamoxifen.
21. (Withdrawn) A negative control serum composition comprising an inactivated immunoglobulin inhibitor that is inactive with respect to the ability to inhibit steroid hormone-responsive cell proliferation in the absence of said steroid hormone; and steroid hormone-depleted blood plasma or serum.
22. (Withdrawn) A method of making a negative control serum composition comprising heating steroid hormone-depleted blood plasma or serum comprising an immunoglobulin inhibitor at about

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

50-60°C for a period of time sufficient to render said inhibitor inactive with respect to the ability to inhibit steroid hormone responsive cancer cell growth *in vitro*.

23. (Withdrawn) The method of claim 22 wherein said period of time is about 90 minutes to about 30 hrs.

24. (Withdrawn) A control serum composition comprising a reactivatable immunoglobulin inhibitor that is inactive with respect to the ability to inhibit steroid hormone-responsive cell proliferation in the absence of said steroid hormone and in the absence of an activating amount of calcium; and steroid hormone-depleted blood plasma or serum.

25. (Withdrawn) The control serum composition of claim 24 containing less than an inhibitor activating amount of calcium ion.

26. (Withdrawn) The control serum composition of claim 24 wherein said immunoglobulin inhibitor is in reactivated form and said composition comprises calcium ion.

27. (Withdrawn) A serum composition comprising steroid hormone free serum and a predetermined amount of at least one immunoglobulin inhibitor chosen from the group consisting of IgA, IgM and IgG.

28. (Withdrawn) A method of making a substantially steroid hormone-depleted serum comprising an inhibitor of steroid hormone responsive cancer cell growth, said method comprising:  
obtaining a non-heat-inactivated fresh or frozen serum specimen;  
performing a first charcoal-dextran extraction on said specimen at about 30-37 °C to yield a first extracted serum; and  
performing a second 30-37°C charcoal-dextran extraction on said first extracted serum to yield a substantially steroid hormone-depleted serum.

29. (Withdrawn) The method of claim 28 comprising performing said first charcoal-dextran extraction on said specimen at about 34°C to yield said first extracted serum and performing a second

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

34°C charcoal-dextran extraction on said first extracted serum to yield said substantially steroid hormone-depleted serum.

30. (Withdrawn) The product of the method of claim 28.

31. (Withdrawn) A method of making a substantially steroid hormone-depleted serum comprising an inhibitor of steroid hormone responsive cancer cell growth, said method comprising:  
obtaining non-heat-inactivated fresh or frozen serum and performing an XAD™ extraction of said serum to provide a substantially steroid hormone-depleted serum.

32. (Withdrawn) The method of making a purified immunoglobulin inhibitor of steroid hormone responsive cancer cell growth comprising:

obtaining a substantially steroid hormone-depleted serum comprising an inhibitor of steroid hormone responsive cancer cell growth;  
loading said depleted serum onto an agarose-based affinity matrix and eluting a fraction comprising said inhibitor;  
loading said fraction onto a phenyl-Sepharose™ matrix and eluting a substantially purified inhibitor pool with a suitable buffer containing ethylene glycol; and  
concentrating said pool to yield a substantially purified inhibitor.

33. (Withdrawn) The product of the method of claim 32.

34. (Currently amended) An *in vitro* assay method for detecting steroid hormone-like cancer cell growth stimulation by a substance of interest, the method comprising:

maintaining a predetermined population of steroid hormone-responsive cells in a steroid hormone-free nutrient medium comprising the medium of claim 45 and comprising a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells also being steroid hormone dependent for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions;

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

determining the cell population in said test mixture after said predetermined period of time, a measurable increase in said cell population indicating a steroid hormone-like cell growth stimulating effect by said substance of interest.

35. (Original) The assay method of claim 34 comprising maintaining serum-free assay conditions.

36. (Original) The assay method of claim 34 comprising adding steroid-hormone depleted serum to said nutrient medium.

37. (Original) The assay method of claim 34 further comprising obtaining non-heat inactivated serum containing said immunoglobulin inhibitors.

38. (Currently amended) The assay method of claim 34 wherein said immunoglobulin inhibitor comprises at least one secretory immunoglobulin chosen from the group consisting of dimeric/polymeric IgA, polymeric IgM and IgG.

39. (Original) The assay method of claim 38 wherein at least one secretory immunoglobulin is chosen from the group consisting of IgG1 and IgG2.

40. (Original) The assay method of claim 39 wherein at least one secretory immunoglobulin is IgG1 $\kappa$ .

41. (Original) The assay method of claim 34 wherein said substance of interest contains or is suspected of containing proteolytic activity, the method comprising selecting an immunoglobulin inhibitor that resists protease degradation.

42. (Original) The assay method of claim 34 wherein said selected immunoglobulin inhibitor comprises IgA2.

43. (Original) The assay method of claim 34 further comprising:

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

maintaining a second predetermined population of steroid hormone-responsive cancer cells in a steroid hormone-free nutrient medium comprising a quantity of inactivated immunoglobulin inhibitor that is incapable of inhibiting cell growth, said cells also being steroid hormone responsive for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and medium, to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions;

determining the cell population in said test mixture after said predetermined period of time, a measurable increase in said cell population indicating a control level cell growth stimulating effect by said substance of interest in the presence of said quantity of inactivated immunoglobulin inhibitor.

44. (Currently amended) A method of detecting a steroid hormone antagonistic substance comprising:

maintaining a predetermined population of steroid hormone responsive cancer cells in a nutrient medium comprising the cell culture medium of claim 45 and comprising a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells also being steroid hormone responsive for *in vivo* proliferation;

adding a defined amount of said substance of interest to said cells and medium;

adding to said cells and medium a defined amount of steroid hormone sufficient to stimulate cell growth in the presence of said inhibitor and in the absence of said substance of interest, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions;

testing said substance of interest for cytotoxic effects on said cells; and

determining the cell population in said test culture after said predetermined period of time, a lack of measurable increase in said cell population not attributable to cytotoxic effects of said substance indicating a steroid hormone antagonistic effect by said substance of interest.

45. (Original) A cell culture medium comprising a basal nutrient fluid substantially devoid of unbound Fe (III) and containing calcium ion.

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

46. (Original) The medium of claim 45 comprising a basal nutrient fluid containing calcium and no more than about 1  $\mu\text{M}$  unbound Fe (III).
47. (Original) The medium of claim 45 further comprising a Fe (III) chelating agent.
48. (Original) The medium of claim 45 wherein said chelating agent is deferoxamine.
49. (Original) The medium of claim 45 further comprising a cell attachment promoting protein.
50. (Original) The medium of claim 49 wherein said protein is fibronectin.
51. (Original) The medium of claim 45 wherein said fluid contains about 1-50 mM calcium ion.
52. (Original) The medium of claim 45 wherein said medium is serum-free.
53. (Original) The medium of claim 45 comprising steroid-hormone depleted serum.
54. (Original) The medium of claim 45 wherein said basal nutrient fluid comprises D-MEM/F-12.
55. (Original) The medium of claim 45 comprising 100 ng/mL to 10  $\mu\text{g}/\text{mL}$  insulin, 0.3 - 10 nM triiodothyronine, 2 - 50  $\mu\text{g}/\text{mL}$  diferric transferrin, 5 - 100  $\mu\text{M}$  ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10  $\mu\text{M}$  deferoxamine, and, optionally, at least one component chosen from the group consisting of 1 - 50 ng/mL EGF, 0.2 - 20 ng/mL aFGF, 5 - 50  $\mu\text{M}$  phosphoethanolamine, 50 - 500  $\mu\text{g}/\text{mL}$  linoleic acid-BSA, 1 - 50  $\mu\text{g}/\text{mL}$  reduced glutathione, 0.5 - 2.0 mM glutamine, 1 - 10  $\mu\text{g}/\text{mL}$  heparin, and 20 - 50  $\mu\text{g}$  (per 35-mm diameter culture dish) human fibronectin.
56. (Currently amended) An *in vitro* method of culturing steroid or thyroid hormone responsive cancer cells or autonomous cancer cells, the method comprising:  
maintaining a predetermined population of steroid or thyroid hormone responsive cells or steroid hormone-independent cancer cells in a steroid hormone-free nutrient medium comprising the

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

medium of claim 45 and a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth of said steroid or thyroid hormone responsive cancer cells in the absence of an inhibition-reversing amount of said steroid or thyroid hormone, to provide an incubation mixture, said steroid or thyroid hormone responsive cells also being steroid or thyroid hormone responsive for proliferation *in vivo* when implanted into a suitable host, and said steroid hormone independent cancer cells also being steroid hormone independent for proliferation *in vivo* when implanted into a suitable host;

optionally, adding an inhibition-reversing amount of said steroid or thyroid hormone to said incubation mixture;

incubating said incubation mixture under cell growth promoting conditions;

optionally, determining the cell population in said reaction mixture after incubation for a predetermined period of time.

57. (Original) An *in vitro* method of detecting a cell growth stimulatory or inhibitory effect of a substance of interest on steroid hormone independent cancer cells, the method comprising:

maintaining a predetermined population of steroid hormone independent cancer cells in a nutrient medium comprising the medium of claim 45, optionally, devoid of said steroid hormone, and, optionally, containing a predetermined quantity of immunoglobulin inhibitor, said steroid hormone independent cells also being steroid hormone independent for proliferation *in vivo* when implanted into a suitable host;

adding a predetermined quantity of said substance of interest to said cells and medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions;

optionally, assessing cytotoxicity of said substance of interest;

determining the cell population in said test mixture after said incubation for said predetermined period of time, a measurable increase in said cell population indicating a cell growth stimulating effect by said substance of interest, and an absence of increase in said cell population, not attributable to cytotoxic effects, indicating a cell growth inhibitory effect by said substance of interest.

58. (Original) An *in vitro* method of detecting an immunoglobulin inhibitor-like cancer cell growth inhibitory effect by a substance of interest comprising:

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

maintaining a predetermined population of steroid hormone responsive cancer cells in a nutrient medium comprising the medium of claim 45, optionally, devoid of said steroid hormone, and, optionally, containing a predetermined quantity of inactivated immunoglobulin inhibitor, said steroid hormone responsive cells also being steroid hormone responsive for proliferation *in vivo* when implanted into a suitable host;

adding a predetermined quantity of said substance of interest to said cells and medium to yield a test mixture;

adding to said test mixture an amount of said steroid hormone that would be sufficient to induce cell growth in the absence of an active immunoglobulin inhibitor;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions;

optionally, assessing cytotoxicity of said substance of interest;

determining the cell population in said test mixture after said predetermined period of time, a measurable increase in said cell population indicating a lack of cell growth inhibitory effect by said amount of said substance of interest, and no increase in said cell population, not attributable to a cytotoxic effect, indicating a cell growth inhibitory effect by said amount of said substance of interest.

59. (Withdrawn) A method of producing a quantity of a biomolecule of interest comprising, in a serum-free nutrient medium comprising the medium of claim 45, culturing a population of cells expressing said biomolecule of interest, harvesting and recovering said biomolecule from said medium.

60. (Withdrawn) The method of claim 59 wherein said biomolecule is chosen from the group consisting of proteins, peptides and polynucleotides.

61. (Withdrawn) The method of claim 59 wherein said protein comprises an antibody.

62. (Original) A method of propagating a virus of interest comprising culturing a population of virus infected cells in a serum-free nutrient medium comprising the medium of claim 45, harvesting and recovering viruses from said medium.

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

63. (Currently amended) An assay kit for detecting *in vitro* steroid hormone reversible steroid hormone-responsive cell growth comprising:

a population of cultured steroid hormone responsive cancer cells that are also steroid hormone responsive for proliferation *in vivo*;

a serum-free defined nutrient cell culture medium comprising the cell culture medium of claim 45 substantially free of unbound Fe (III) and comprising calcium ion; and

an immunoglobulin inhibitor composition.

64. (Withdrawn) The kit of claim 63 wherein said steroid hormone responsive cancer cells are MTW9/PL2 rat mammary tumor cells.

65. (Withdrawn) The kit of claim 63 wherein said immunoglobulin inhibitor composition comprises a substantially steroid hormone-depleted serum prepared by a method comprising:

obtaining non-heat inactivated fresh or frozen serum specimen;

performing a first charcoal-dextran extraction on said specimen at about 30-37°C to yield a first extracted serum;

performing a second 30-37°C charcoal-dextran extraction on said first extracted serum to yield a substantially steroid hormone-depleted serum, and

optionally, loading said depleted serum onto an agarose-based affinity matrix and eluting a fraction comprising an immunoglobulin inhibitor, and

optionally, loading said fraction onto a phenyl-Sepharose™ matrix, eluting a substantially purified immunoglobulin inhibitor pool with a suitable buffer containing ethylene glycol, and concentrating said pool to yield a substantially purified immunoglobulin inhibitor.

66. (Withdrawn) The kit of claim 65 wherein said method comprises performing a first charcoal-dextran extraction on said specimen at about 34°C to yield a first extracted serum and performing a second 34°C charcoal-dextran extraction on said first extracted serum to yield a substantially steroid hormone-depleted fraction comprising an immunoglobulin inhibitor.

67. (Withdrawn) The kit of claim 63 wherein said immunoglobulin inhibitor composition comprises at least one secretory immunoglobulin chosen from the group consisting of IgA, IgM and IgG and a carrier.

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

68. (Withdrawn) The kit of claim 63 further comprising at least one assay additive chosen from the group consisting of steroid hormones, and agonists and antagonists thereof.

69. (Withdrawn) The kit of claim 63 wherein said immunoglobulin inhibitor composition comprises a substantially steroid hormone-depleted serum prepared by a method comprising obtaining a non-heat inactivated fresh or frozen serum specimen and performing a XAD-4™ extraction of said specimen to provide a substantially steroid hormone-depleted serum containing an immunoglobulin inhibitor.

70. (Withdrawn) A method of measuring the amount of steroid hormone reversible inhibitor of steroid hormone responsive cell growth in a body fluid sample comprising:

obtaining a body fluid sample;

depleting steroid hormone from said sample;

isolating an immunoglobulin inhibitor fraction from said steroid hormone depleted sample; and

assaying said steroid hormone-depleted immunoglobulin inhibitor fraction for steroid hormone reversible inhibition of steroid hormone responsive cell growth in a predetermined population of cultured cells maintained in nutrient medium under cell growth promoting culture conditions, said cells being steroid hormone responsive for *in vivo* proliferation when implanted into a suitable host, a measurable increase in inhibition of cell growth with increasing concentration of immunoglobulin inhibitor fraction, at a defined concentration of steroid hormone in said medium, being indicative of the amount of inhibitor in said body fluid sample.

71. (Withdrawn) The method of claim 70 wherein said depleting comprises performing a first charcoal-dextran extraction on said body fluid sample at about 30-37°C to yield a first extracted fluid; performing a second 30-37°C charcoal-dextran extraction on said first extracted fluid to yield a substantially steroid hormone-depleted immunoglobulin inhibitor fraction comprising at least one immunoglobulin chosen from the group consisting of IgA, IgM and IgG.

72. (Withdrawn) The method of claim 71 wherein said body fluid is chosen from the group consisting of serum, plasma, urine, seminal fluid, milk, colostrum and mucus.

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

73. (Original) An *in vitro* assay method for detecting an immunoglobulin inhibitor of steroid hormone responsive cell growth in a sample of interest, the method comprising:

maintaining a predetermined population of steroid hormone-responsive culture cells in a nutrient medium, said cells also being steroid hormone dependent for proliferation *in vivo* when implanted into a suitable host;

adding a quantity of steroid hormone to said medium sufficient to stimulate proliferation of said cells under cell growth promoting culture conditions;

adding a predetermined quantity of said sample of interest to said medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting culture conditions;

optionally, testing said sample for cytotoxic effects on said cells;

determining the cell population in said test mixture after said predetermined period of time, a measurable decrease in said cell population not attributable to cytotoxic effects indicating inhibition by said amount of sample of steroid hormone responsive cell growth.

74. (Original) The assay method of claim 73 further comprising:

adding to said test mixture an amount of said steroid hormone in excess of the minimum amount necessary to maximally stimulate proliferation of said cells; and

determining the cell population of said test mixture after said predetermined period of time, a measurable increase in said cell population indicating reversal by said excess amount of steroid hormone of steroid hormone responsive cell growth inhibition.

75. (Original) An *in vitro* cell culture model for predicting an *in vivo* steroid hormone-responsive cancer cell growth effect of a defined stimulus, said model comprising: steroid hormone-responsive cancer cells maintained in a growth medium containing a basal nutrient fluid substantially free of unbound Fe (III), containing calcium ion, and containing an amount of steroid hormone reversible immunoglobulin inhibitor sufficient to arrest cancer cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said inhibitor chosen from the group consisting of IgA, IgM and IgG, and combinations thereof, said cells also being steroid hormone responsive for proliferation *in vivo*.

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

76. (Original) The model of claim 75 wherein said nutrient medium is serum-free.
77. (Original) The model of claim 76 wherein said nutrient medium contains steroid hormone depleted serum or plasma.
78. (Original) The model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of MTW9/PL2 (rat mammary cancer), T47D (human breast carcinoma), MCF-7 (human breast carcinoma), MCF-7A (human breast carcinoma), MCF-7K (human breast carcinoma), LNCaP (human prostatic carcinoma), ZR-75-1 (human prostatic carcinoma), H-301 (Syrian hamster kidney tumor), GH<sub>1</sub> and GH<sub>3</sub> (rat pituitary tumor), GH<sub>4</sub>C<sub>1</sub> (rat pituitary tumor), and HT-29 (human colonic cancer).
79. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of T47D (human breast carcinoma), MCF-7 (human breast carcinoma), MCF-7A (human breast carcinoma) and MCF-7K (human breast carcinoma) and said medium comprises 100 ng/mL to 10  $\mu$ g/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50  $\mu$ g/mL diferric transferrin, 5 - 100  $\mu$ M ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10  $\mu$ M deferoxamine, 1 - 50 ng/mL EGF, 5 - 50  $\mu$ M phosphoethanolamine, 50 - 500  $\mu$ g/mL linoleic acid-BSA, 1 - 50  $\mu$ g/mL reduced glutathione, 0.5 - 2.0 mM glutamine, and 20 - 50  $\mu$ g per 35-mm diameter culture dish human fibronectin.
80. (Original) The *in vitro* cell culture model of claim 75 wherein said medium comprises 500 ng/mL insulin, 0.3 nM triiodothyronine, 10  $\mu$ g/mL diferric transferrin, 50  $\mu$ M ethanolamine, 500  $\mu$ g/mL bovine serum albumin (BSA), 20 ng/mL selenium, 5  $\mu$ M deferoxamine, 20 ng/mL EGF, 5  $\mu$ M phosphoethanolamine, 2.0 mM glutamine, 150  $\mu$ g/mL linoleic acid-BSA, 20  $\mu$ g/mL reduced glutathione, and 25  $\mu$ g per 35-mm diameter culture dish human fibronectin.
81. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of LNCaP (human prostatic carcinoma), ZR-75-1 (human prostatic carcinoma), H-301 (Syrian hamster kidney tumor), and HT-29 (human colonic cancer) cells, and said medium comprises 100 ng/mL to 10  $\mu$ g/mL insulin, 0.3 - 10

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

nM triiodothyronine, 2 - 50 µg/mL diferric transferrin, 5 - 100 µM ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10 µM deferoxamine, 1 - 50 ng/mL EGF, 0.2 - 20 ng/mL aFGF, 1 - 10 µg/mL heparin, and 20 - 50 µg per 35-mm diameter culture dish human fibronectin.

82. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of LNCaP (human prostatic carcinoma), ZR-75-1 (human prostatic carcinoma), H-301 (Syrian hamster kidney tumor), and HT-29 (human colonic cancer) cells, and said medium comprises 10 µg/mL insulin, 1 nM triiodothyronine, 10 µg/mL diferric transferrin, 50 µM ethanolamine, 1.0 mg/mL bovine serum albumin (BSA), 10 ng/mL selenium, 10 µM deferoxamine, 20 ng/mL EGF, 10 ng/mL aFGF, 7.5 µg/mL heparin, and 20 µg per 35-mm diameter culture dish human fibronectin.

83. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are MTW9/PL2 rat mammary cancer cells, and said medium comprises 100 ng/mL to 10 µg/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50 µg/mL diferric transferrin, 5 - 100 µM ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10 µM deferoxamine, 1 - 50 ng/mL EGF, 5 - 50 µM phosphoethanolamine, 50 - 500 µg/mL linoleic acid-BSA, 1 - 50 µg/mL reduced glutathione, 0.5 - 2.0 mM glutamine, and 1 - 10 µg/mL heparin.

84. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are MTW9/PL2 rat mammary cancer cells, and said medium comprises 10 µg/mL insulin, 0.3 nM triiodothyronine, 10 µg/mL diferric transferrin, 50 µM ethanolamine, 500 µg/mL bovine serum albumin (BSA), 20 ng/mL selenium, 2 - 10 µM deferoxamine, 20 ng/mL EGF, 5 - 50 µM phosphoethanolamine, 150 µg/mL linoleic acid-BSA, 20 µg/mL reduced glutathione, 2.0 mM glutamine, and 1 - 10 µg/mL heparin.

85. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of GH<sub>1</sub>, GH<sub>3</sub> and GH<sub>4</sub>C<sub>1</sub> rat pituitary tumor cells, and said medium comprises 100 ng/mL to 10 µg/mL insulin, 0.3 - 10 nM

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

triiodothyronine, 2 - 50 µg/mL diferric transferrin, 5 - 100 µM ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, and 2 - 10 µM deferoxamine.

86. (Currently amended) The *in vitro* cell culture model of claim 66-75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of GH<sub>1</sub>, GH<sub>3</sub> and GH<sub>4C1</sub> rat pituitary tumor cells, and said medium comprises 10 µg/mL insulin, 1 nM triiodothyronine, 10 µg/mL diferric transferrin, 10 µM ethanolamine, 500 µg/mL bovine serum albumin (BSA), 10 ng/mL selenium, and 10 µM deferoxamine.

87-94. (Canceled)

95. (Currently amended) ~~The method of claim 34 A method of detecting an estrogenic substance comprising:~~

maintaining a predetermined population of estrogen responsive cancer cells in a steroid hormone-free nutrient medium comprising a quantity of immunoglobulin inhibitor sufficient to inhibit cancer cell growth in the absence of an inhibition-reversing amount of estrogen, said cells also being estrogen responsive for proliferation *in vivo* when implanted into a suitable host;

adding a defined amount of said substance of interest to said cells and medium, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions; and

determining the cell population in said test culture after said predetermined period of time, a measurable increase in said cell population indicating an estrogen-like cell growth stimulating effect by said substance of interest, whereby an estrogenic substance is detected.

96. (Original) The method of claim 95 further comprising testing said substance of interest for binding to estrogen receptor gamma.

97. (Original) The method of claim 95 further comprising testing said substance of interest for cytotoxic effects on said cells.

Appl. No. 09/852,958

Amendment Dated June 15, 2004

Reply to Office Action of October 3, 2003

98. (Original) The method of claim 95 further comprising selecting estrogen responsive cancer cells containing estrogen receptor gamma.

99. (Currently amended) The method of claim 44 A method of detecting an anti-estrogenic substance comprising:

maintaining a predetermined population of estrogen responsive cancer cells in a nutrient medium comprising a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of estrogen, said cells being capable of growing *in vivo*;

adding a defined amount of said substance of interest to said cells and medium;

adding a defined amount of an estrogen sufficient to stimulate cell growth in the presence of said inhibitor and in the absence of said substance of interest to said cells and medium, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions;

testing said substance of interest for cytotoxic effects on said cells; and

determining the cell population in said test culture after said predetermined period of time, a lack of measurable increase in said cell population not attributable to cytotoxic effects of said substance indicating a steroid hormone antagonistic effect by said substance of interest, whereby an anti-estrogenic substance is detected.

100. (Original) The method of claim 99 further comprising testing said substance of interest for binding to estrogen receptor gamma.

101. (Original) The method of claim 99 further comprising testing said substance of interest for cytotoxic effects on said cells.

102-108. (Canceled)

109. (New) The method of claim 34 wherein said steroid hormone-free nutrient medium comprises no more than about 1 $\mu$ M unbound Fe(III).

110. (New) The method of claim 34 wherein said medium comprises a Fe (III) chelating agent.

Appl. No. 09/852,958  
Amendment Dated June 15, 2004  
Reply to Office Action of October 3, 2003

111. (New) The method of claim 34 wherein said medium comprises a cell attachment promoting protein.

112. (New) The method of claim 34 wherein said medium contains about 1-50 mM calcium ion.

113. (New) The method of claim 34 wherein said basal nutrient fluid comprises D-MEM/F-12.

114. (New) The method of claim 34 wherein said medium comprises 100 ng/mL to 10  $\mu$ g/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50  $\mu$ g/mL diferric transferrin, 5 - 100  $\mu$ M ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10  $\mu$ M deferoxamine, and, optionally, at least one component chosen from the group consisting of 1 - 50 ng/mL EGF, 0.2 - 20 ng/mL aFGF, 5 - 50  $\mu$ M phosphoethanolamine, 50 - 500  $\mu$ g/mL linoleic acid-BSA, 1 - 50  $\mu$ g/mL reduced glutathione, 0.5 - 2.0 mM glutamine, 1 - 10  $\mu$ g/mL heparin, and 20 - 50  $\mu$ g (per 35-mm diameter culture dish) human fibronectin.

115. (New) The method of claim 44 wherein said nutrient medium comprises no more than about 1  $\mu$ M unbound Fe(III).

116. (New) The method of claim 44 wherein said nutrient medium comprises a Fe (III) chelating agent.

117. (New) The method of claim 44 wherein said nutrient medium comprises a cell attachment promoting protein.

118. (New) The method of claim 44 wherein said nutrient medium contains about 1-50 mM calcium ion.

119. (New) The method of claim 44 wherein said nutrient medium is serum-free.

120. (New) The method of claim 44 wherein said nutrient medium comprises steroid-hormone depleted serum.

**Appl. No. 09/852,958**  
**Amendment Dated June 15, 2004**  
**Reply to Office Action of October 3, 2003**

121. (New) The method of claim 44 wherein said nutrient medium comprises D-MEM/F-12.

122. (New) The method of claim 44 wherein said nutrient medium comprises:

100 ng/mL to 10  $\mu$ g/mL insulin,  
0.3 - 10 nM triiodothyronine,  
2 - 50  $\mu$ g/mL diferric transferrin,  
5 - 100  $\mu$ M ethanolamine,  
0.2 - 5.0 mg/mL bovine serum albumin (BSA),  
5 - 20 ng/mL selenium,  
2 - 10  $\mu$ M deferoxamine, and,

optionally, at least one component chosen from the group consisting of

1 - 50 ng/mL EGF,  
0.2 - 20 ng/mL aFGF,  
5 - 50  $\mu$ M phosphoethanolamine,  
50 - 500  $\mu$ g/mL linoleic acid-BSA,  
1 - 50  $\mu$ g/mL reduced glutathione,  
0.5 - 2.0 mM glutamine,  
1 - 10  $\mu$ g/mL heparin, and  
20 - 50  $\mu$ g human fibronectin (per 35-mm diameter culture dish).